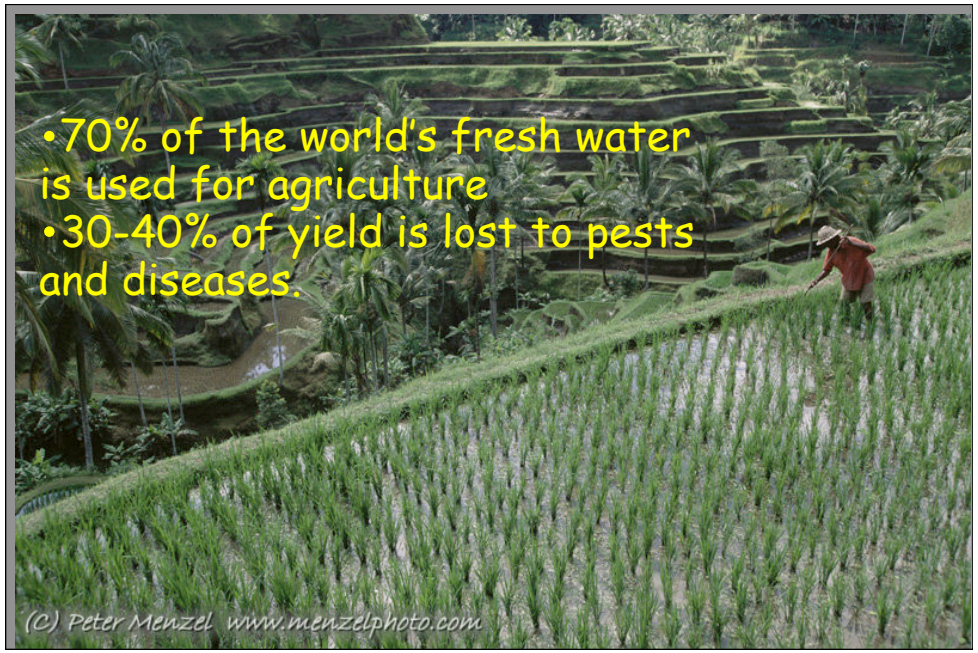
Yields can be comparable to conventional farms depending on the crop and location, but for some key crops such as rice, yields are often lower.

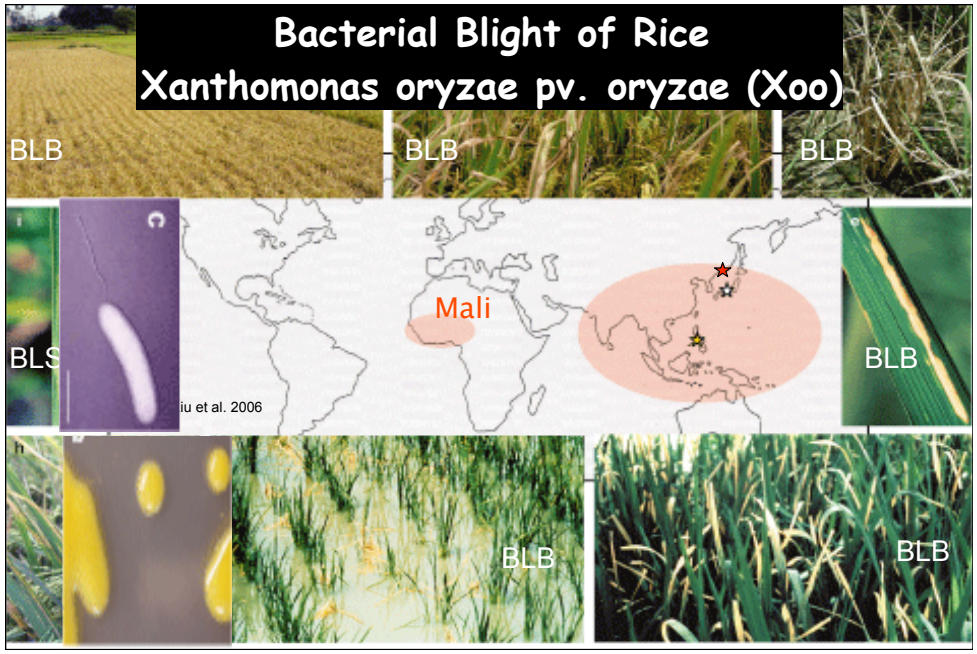
Some pests are difficult to control using organic methods

Mal.mw.21.xxsNatomo family dinner of rice porridge cooks on the hearth over a wood fire. Published in Material World, Meals of the World gallery, page 176. The Natomo family (Soumana Natomo, his two wives—Pama Kondo and Fatoumata Toure—and 7 children) lives in two mud brick houses in the village of Kouakourou, Mali, on the banks of the Niger River. {{They are grain traders and own a mango orchard. According to tradition Soumana is allowed to take up to four wives; he has two. Wives Pama and

Planting rice near Alexandria, Egypt.



man cultives his terrace rice fields near Ubud at Penatahan in Bali, Indonesia.



THirty years ago, plant breeders discovered an unsual strain of a wild species of rice in Mail that was resistant to all strains tested. never showed infection in the field.

We are using the Xoo/rice interaction to understand how plants **sensee and respond to pathogens**

To understand the plant response to microbial infection, my laboratory is studying the interaction of gram negative bacterium, Xoo with its host rice

The Gram negative bacteria, Xoo causes Bacterial blight disease, the most serious bacterial disease of rice in in Asia and Africa. BEcause rice is a staple for halfs the words population, develping methods to contorl this disease is important.

BB was deiscovere din 1884 in Fukuoka Prefecture of Japan

Resistance to diverse Xoo strains was identified in the wild species Oryza longistaminata

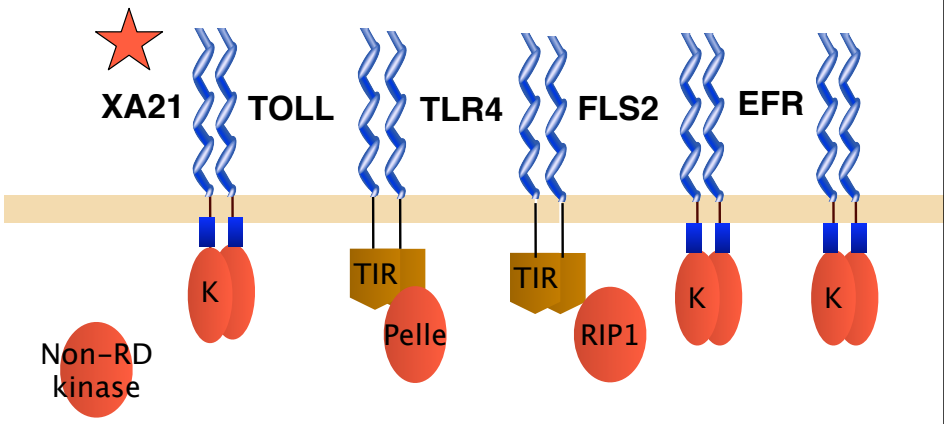
Transgenic plants expressing the Xa21 gene confer broad-spectrum resistance to Xoo

Song et al, 1995, Science 270:1804

It appeared that A21 recognzied a conserved component in all the diverse starins taht had been tested. Thus we were quite interested in isolating this gene. We carried otu a map based cloning approach to isoalte the gene.

The rice leaves on the left were infected with Xoo using scissors dipped in bacteria. after 2 weeks lesions develop the length of the leaf...

Xa21 confers resistance to the gram negative bacteria Xoo.. On the right is a pair of leaves from a transgenic line carrying XA21. The pathogen triggers rapid cell death at the site of infection and growth is restricted.

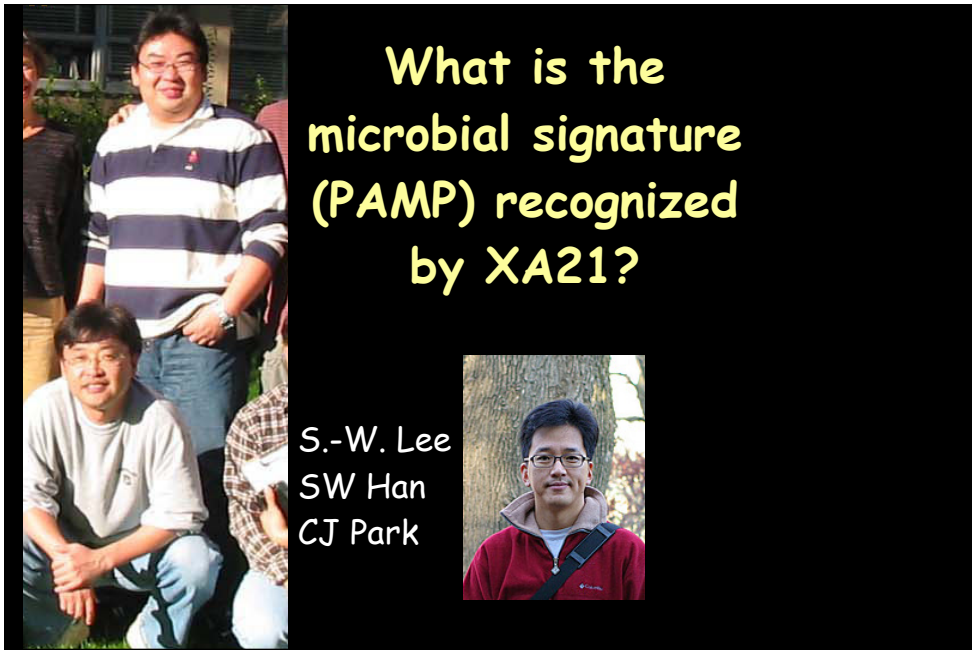


XA21 encodes a receptor kinase with an EC LRR domain, transmemebrane, JM and intracellular kinase domain.

Subsequent discoveries revealed that animals and other plants also carry this type of protien, called a PRR.

We surveyed the yeast, fly, worm, human, Arabidopsis, and rice kinomes (3,723 kinases) and found that despite the small number of non-RD kinases in these genomes, **kinases known or predicted to function in PRR signaling fall into the non-RD class or associate iwth a non-RD kinase**

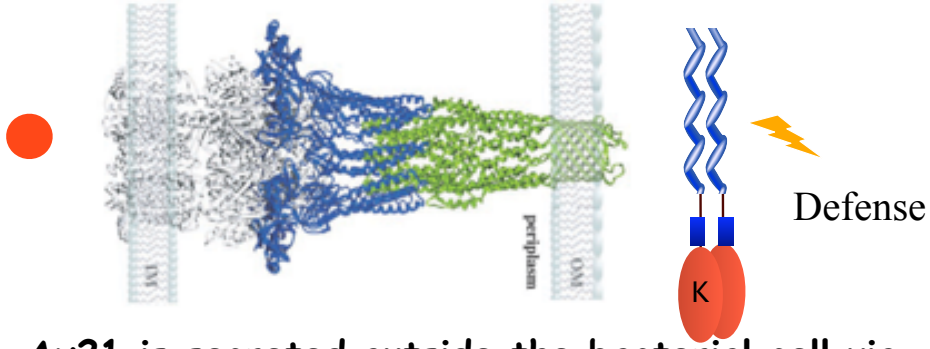
For example. the Drosophila Toll protein consists of (LRRs) in the extracellular (EC) domain and is required for



To identify the PAMP recognized by Xa21, we looked at the omlecules taht might be secreted by the RAXABC styesm

What is secretd by RaxAB-C? This is an active area of research in my lab.

A 17 aa sulfated peptide, called Ax21 (Activator of Xa21-mediated immunity), binds XA21 and triggers Xa21-mediated immunity

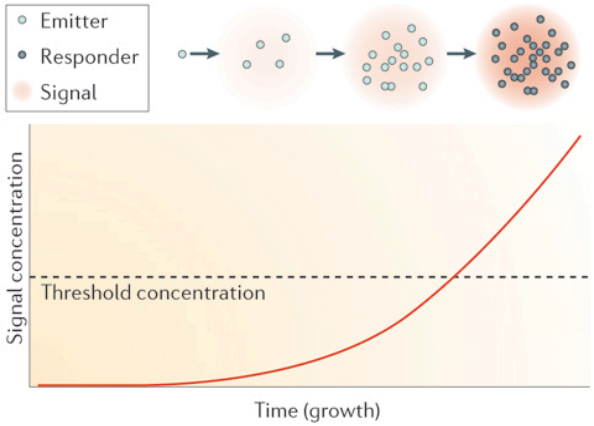


Ax21 is secreted outside the bacterial cell via the RaxABC TypeI secretion system

In its simplest form, cell?cell signalling results from the production of signalling molecules by emitter cells and their accumulation in the surrounding environment. At some threshold concentration, the signalling molecules bind to receptors on or in the bacterial cell, leading to changes in gene expression in the responding cell. For intraspecies quorum sensing, the emitter and responder are usually the same cells, as illustrated here. Often, but not always, the genes that are involved in synthesis and response activate their own expression ? explaining the term autoinducer. A signalling molecule is considered to act at low concentrations and not to be involved in primary metabolism.

- **Certain traits are only expressed when bacteria are**

Gram positive bacteria use Type I secreted peptides as quorum sensing factors



Keller *et al. Nature Reviews Microbiology*; published online 27 February 2006 | doi:10.1038/nrmicro1383

At some threshold concentration, the signaling molecule binds to receptors on the bacterial cell, leading to changes in gene expression.

In its simplest form, cell?cell signalling results from the production of signalling molecules by emitter cells and their accumulation in the surrounding environment. At some threshold concentration, the signalling molecules bind to receptors on or in the bacterial cell, leading to changes in gene expression in the responding cell. For intraspecies quorum sensing, the emitter and responder are usually the same cells, as illustrated here. Often, but not always, the genes that are involved in synthesis and response activate their own expression ? explaining the term autoinducer. A signalling molecule is considered to act at low concentrations and not to be involved in primary metabolism.

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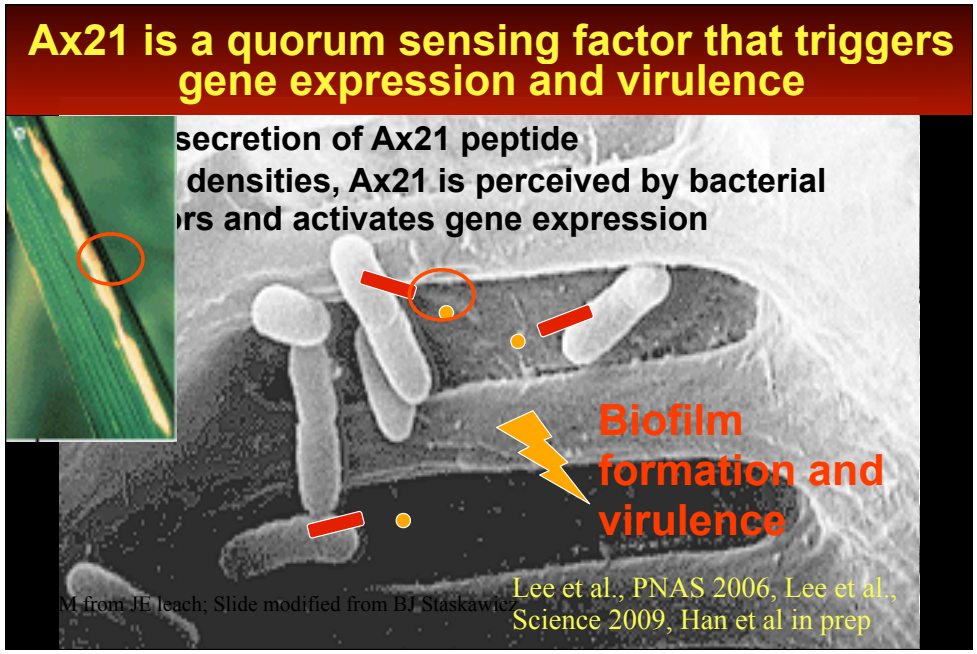
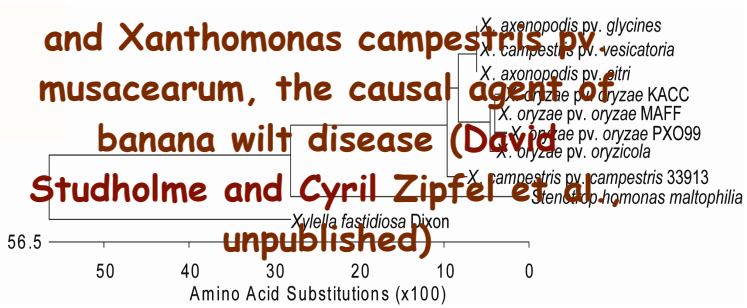


A solitary bacterium cannot form a biofilm by itself - it must wait until a group of bacteria has gathered. With no fingers to count on, how do bacteria know when there are enough others nearby? Bacteria are equipped with molecular

Ax21 is highly conserved in all Xanthomonas species (92-100%), Xylella fastidiosa (48%) and the human pathogen Stenotrophomonas maltophilia (61%)

and Xanthomonas campestris pv. musacearum, the causal agent of banana wilt disease (David Studholme and Cyril Zipfel et al., unpublished)



Sang-Wook Han
Lee et al., Science, 2009, 326:850

ax21 is present in all sequenced *Xanthomonas* species (92-100% identity), in *Xylella fastidiosa (*48% identity), the causal agent of Pierce's disease on grapes, and in the human pathogen, *Stenotrophomonas maltophilia (*61% identity. *S. maltophilia* is a gram-negative bacterium that is widespread in the environment and that has become important in the last 15 years as an emerging opportunistic pathogen associated with nosocomial colonization and infection {Lee, 2009 #2}{Ryan, 2008 #25}.

Thus Ax21 is a PAMP that is broadly conserverd within a class of microbes

•100 million people rely on banana as a staple food source.
•Banana wilt attacks all banana varieties causing complete crop loss.
•Conventional breeding not an option

caused by *Xanthomonas campestris* pv. *musacearum*

First reported in Uganda in 2001.
The disease has also been reported in DR Congo,

•Can we develop new strategies to control this disease?

-Direct transfer of XA21 or engineered variants into banana
-New strategies to disrupt bacterial cell to cell communication

caused by *Xanthomonas campestris* pv. *musacearum*

First reported in Uganda in 2001.
The disease has also been reported in DR Congo,

man cultives his terrace rice fields near Ubud at Penatahan in Bali, Indonesia.

Climate change will result in more flooding

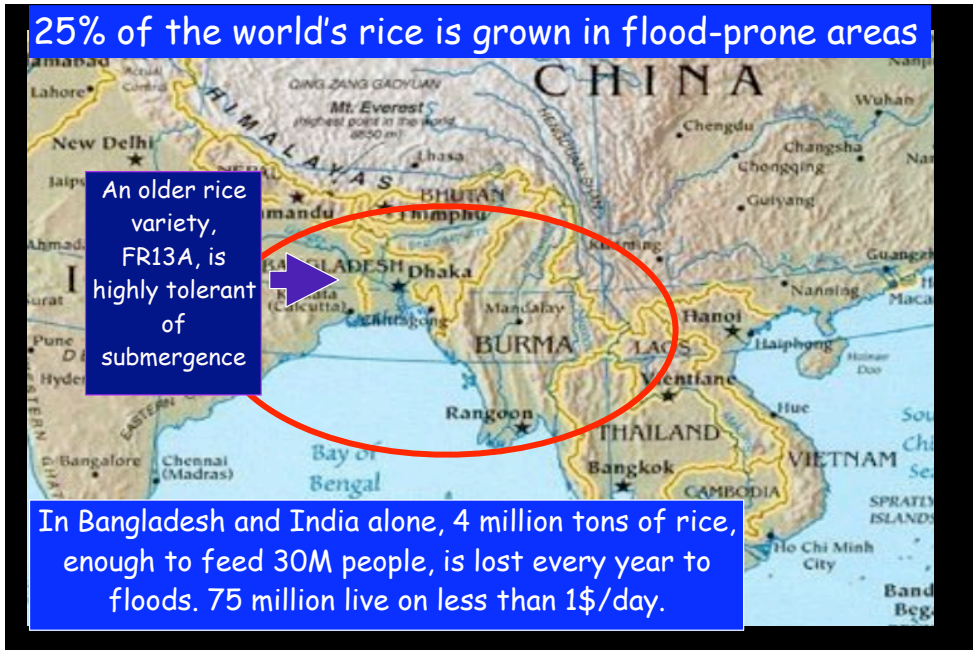
- Rapid melting of the Himalayan glaciers will cause river systems to experience shorter and more intense seasonal flow and more flooding—thus affecting large tracks of agricultural land.

2008, Intergovernmental Panel on Climate Change

Sea level rise will be most devastating for countries such as Bangladesh that are low-lying and highly populated.

The rapid melting of the

Large areas of Bangladesh already flood on an annual basis and are likely to be submerged completely in the future, leading to a substantial loss of

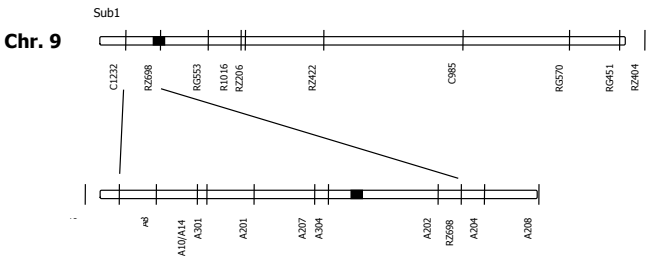


Nearly all rice varieties die within 1 week of submergence

1/4 of the global rice cropland is prone to flooding (10-15 M ha).

Submergence tolerance is controlled by the Sub1 QTL

- Submergence1 (Sub1) QTL mapped to Chr. 9;
- Sub1 controls ~70% variation in submergence tolerance



Xu and Mackill, 1996

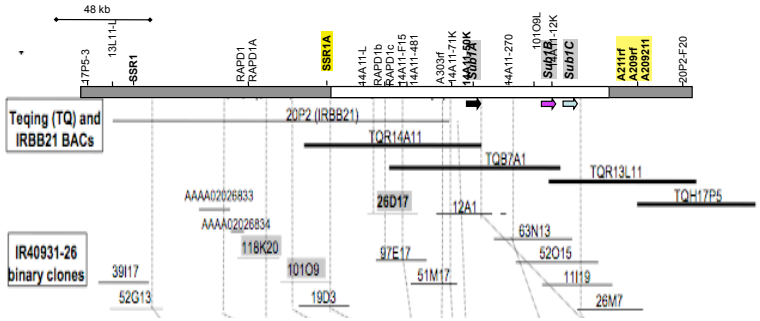
Xu, Xu, Mackill and Ronald. 2000

The sub1 locus was mapped to 0.0 cM on chromosme 9 usig a mapping population o (Dx202) of 4022 plants developed from the hbridizatio of a tolerant indica derivative of Fr13A (IR40931-2) and intolerant japanocia M202. Physical overage was obtained with 5 overlapping BAC clones from intolerant indica (wing) and a nerly coplete contig of 13 clones from tolerant IR40931-26. The sub1 region bordered by the markers CR25K and SSR1A, pyscially span 182 kb. This region includes 3 ERFs (ten non-ERFs, 6 re ypothetical). The corresponding region in nipponbare spans 142 kb and is rearranged. Sub1 A is absent from Nipponbare. Recominatino was supporeesed in this region in this mapping population as revealed by a 10.7 fod higher than average recombination ration (3,030 Kb/cm Vs. 282 kb/cm for the entire genome). This could reflect the prosimity fo the sub1 locus to the centromere and/or the presence of genomic rearrangemnts that have altered continuti int his region in the two rice subspecies.

Isolation of the Sub1 QTL at UC Davis

P. Canlas K Xu X Xu

A contiguous set of BAC and biBAC clones spans the 182 kb Sub1 region



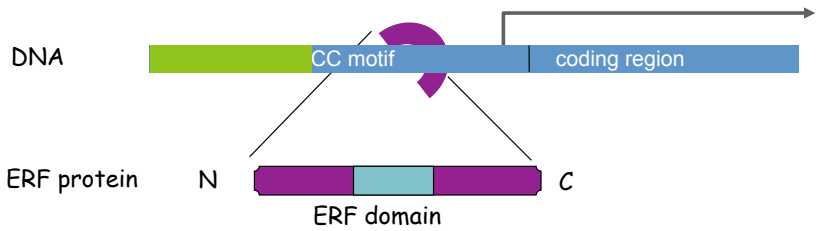
The Sub1 region contains 13 genes including a variable cluster of ethylene response factor (ERF)-domain transcription factor genes and >50% retrotransposon- related sequences

Xu et al., Nature. 2006. 442:705-708

Physical coverage of this region was obtained with five overlapping bacterial artificial chromosome (BAC) clones derived from submergence-intolerant indica rice varieties and a nearly complete contig of 13 binary clones from IR40931-26. The Sub1 region, bordered by the markers d SSR1A and CR25K (???), physically spans over 182 kilobases (kb).

The 182 kb i nt er val encodes three genes cont ai ni ng et hyl ene- response-factor (ERF) like genes that were designated Sub1A

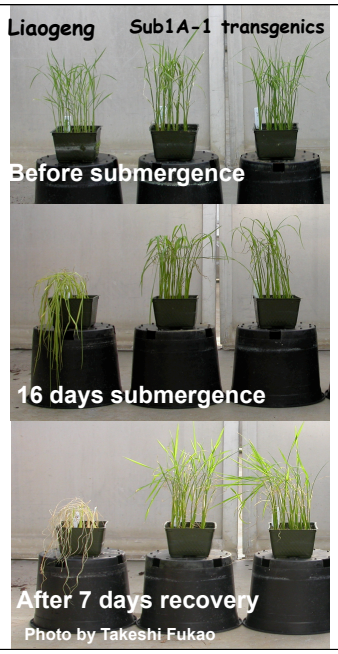
SUB1 proteins are members of the Ethylene Responsive Family of transcription factors



- ERFs are known regulators of abiotic and biotic stress responses

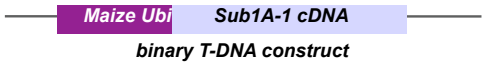
slide courtesy of J. Bailey-Serres

- ERFs are known regulators of abiotic and biotic stress responses
- ERF DNA binding proteins regulate gene transcription
- ~106 ERFs in rice
- all possess a conserved 56



Ectopic expression of Sub1A-1 is sufficient to confer submergence tolerance to highly intolerant japonica

***Ubi:*Sub1A-1**



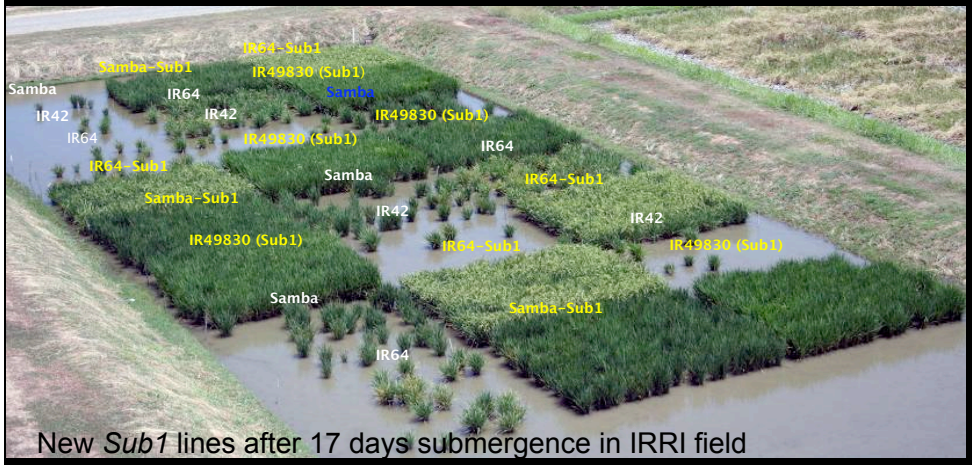
Xu et al. Nature, 2006

Plant submergence due to complete flooding restricts diffusion of oxygen and carbon dioxide by 10^4-fold, which inhibits aerobic respiration and photosynthesis

Responses that can enhance survival of transient

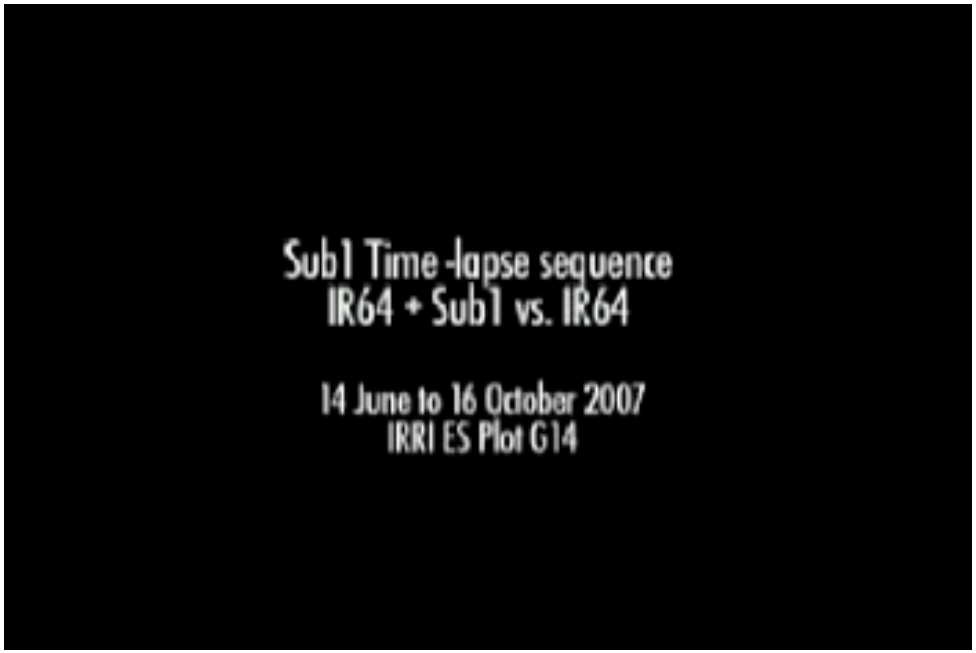
- *Sub1* QTL: 3 ERF genes (*Sub1A*, *Sub1B*, and *Sub1C*; Xu et al., 2006)
- Facilitate the development of recombinant and gene-based markers
  - *Enable genetic study within Sub1 region*
  - *Facilitate precise-MAB*
  - *Minimize linkage drag*
  - *Eliminate the need for phenotyping*
  - *Facilitate more efficient selection in combination with different traits (salinity, flooding during germination, etc)*

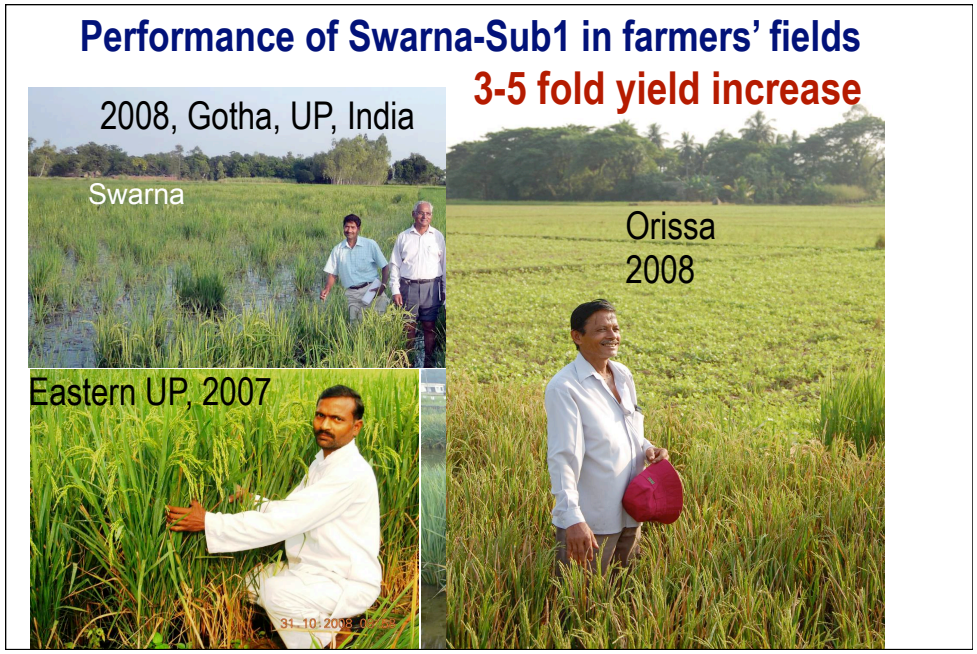
Sub1A is sufficient to confer tolerance to nearly all intolerant varieties (5-6 fold yield increase)



New *Sub1* lines after 17 days submergence in IRRI field

Xu et al., Nature 2006; Slide Courtesy of D. Mackill



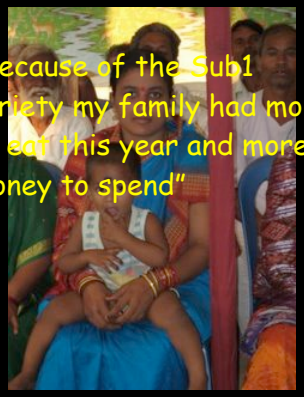


Three-quarters of the world's poorest people get their food and income by farming small plots of land. So if we can make small-holder farming more productive and more profitable, we can have a massive impact on hunger and nutrition and poverty.

her job is seeding the rice and transplanting, She likes the swarna variety best.

She also said it tastes very good.

In flooded fields in India, villagers were able to harvest more rice for their families



I wil be in this field next week. NOt a good week for C diet. And it doesn't get any more real than northern Bangladesh, where the vast Brahmaputra River often spills over its banks and into the fields of struggling rice farmers. The people of Bangladesh get about two-thirds of their total calories from rice

Dr. Md Abdul Mazid, of the Bangladesh Rice Research Institute, stands in a field of flood-resistant rice.

Ocrober 2007 NPR:

The field was under water for eight days. Gobindra says that usually after eight days, the crop would be damaged. But the sub-1 rice is still thriving… Gobindra says his neighbors are amazed by what they've seen in his paddy. Standing in a semi-circle in front of a shed made of

Dramatic advancements in plant genetics

Arabidopsis genome sequence:
2000: 7 years, $70 million, 500 people
2011: 2-3 minutes, $99

source: Joe Ecker

With this information we can develop improved crops for California and the world

Goal: Combine diverse classes of functional data into a composite network that is larger and more accurate than that from any individual dataset.

Analyzed ~50 millions data points

23 types of data sets from 5 different species

~45% of the rice genome integrated into a gene network

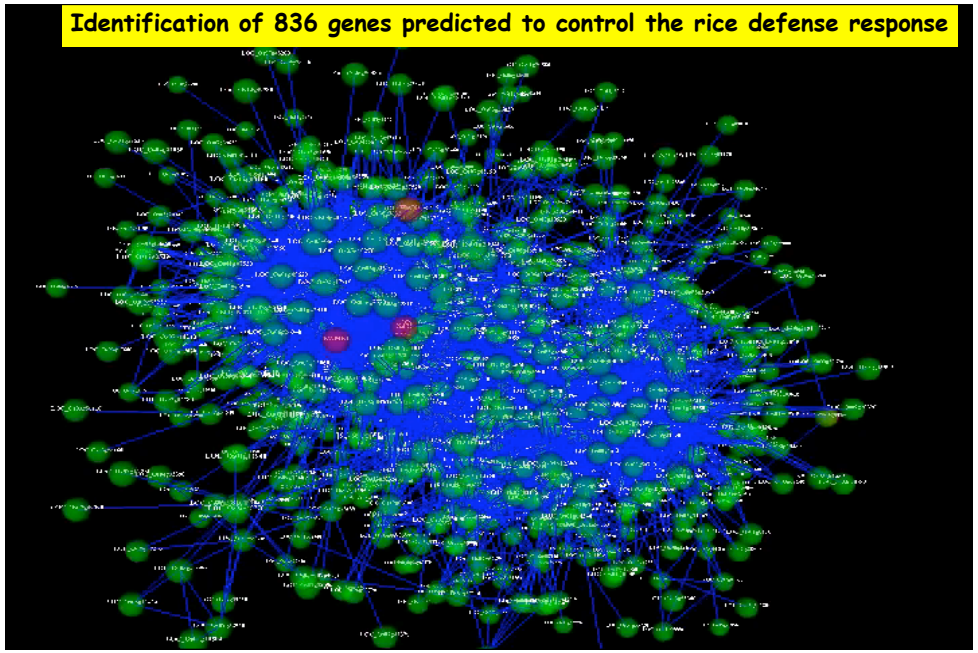
Lee, Marcotte and Ronald, Nature Genetics, Under Review
Seo, Lee and Ronald, Nature Genetics, Under Review

Marcotte group applied probabilistic functional gene networks to yeast and worm to identify new genes in involved in cellular responses.

In particular, RNAi-mediated phenotypic analysis in worm revealed that 20% (10 out 50) in computational core interactors and 5% (6 out of 124) in computational non-core intreractors from network are functionally validated in retinoblastoma tumor suppressor pathway.

Instead of fully modeling the mechanistic details of gene relations—for example, physical interactions—marcotte modelsl "functional coupling", a high-level abstraction of gene relationships.

). Thus, a major aspect of reconstructing a functional gene network is to quantify the quality of the data, especially the strength of functional inferences that can be drawn (e.g., using a Bayesian probabilistic approach that Marcotte lab developed [38]), then to



We are now using advanced computational and proteomics tools to identify, with a high degree of fidleity, all the genes that control response to stress. Each ball in this image represents a protein ccontrolling these responses and we are able to identify networks of these protesin that are likely key regulators. Whereas it took us many years to clone and validate the function f 2 key genes (Sub1a and Xa21) using traditional approaches we now can do this work much faster. In the last couple years we have identified 2300 genes and validated 25 using the newest genomic information modern apporaches. Given the large genomes of most crop species (generally 30,000 – 50,000 genes) and their long reproductive cycles (often several months), such computational based coupled with rigrourous genetic analuysis offer s arapid route for focusing crop engineering efforts on the small sets of genes that are deemed most likely to affect the traits of interest.

Imagine...the world your children will live in 50 years from now if we make no changes to our agricuttularl prodcition

If we continue with current farming practices, vast amounts of wilderness will be lost, millions of birds and billions of insects will die, farm workers will be at increased risk for disease, and the public will lose billions of dollars as a consequence of environmental degradation. Clearly, there must be a better way to resolve the need for increased food production with the desire to minimize its impact.

Raoul and I start from this point, recognizing that there are problems with agriculture that need to be solved.